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SUSCEPTIBILITY OF MIL-L-23699 (ACFT TURBO-SHAFT ENGINE, SYNTHETIC LUBE OIL) AND MIL-L-7808 (LUBRICATING OIL ACFT TURBINE ENGINE, SYNTHETIC BASE) OILS TO MICROBIAL ATTACK

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Army Natick Laboratories
Natick, Massachusetts

April 1973

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FUNGI			6			
FUNGAL GROWTH			6			
DRAINAGE			7			
AIRCRAFT ENGINE OILS			9		9	
GAGES (OIL)			7,9			
AIRCRAFT GAGES			7,9			
CANAL ZONE			9			
LUBRICANTS					6	
TRANSMISSION FLUID					6	
MOTOR OILS					6	
SUSCEPTIBILITY (PHYSIOLOGY)					7	
TOLERANCES (PHYSIOLOGY)					7	
MICROBIAL					0	
RESISTANCE					7	
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by

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April 1973

1. PIONEERING RESEARCH LABORATORY

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ABSTRACT

A 6-month tropic service test of the OH-58A Helicopter System conducted at Fort Clayton, Canal Zone revealed that the tail rotor transmission oil level sight gage did not indicate the correct oil level because fungal growth plugged the sight gage drain holes. A sample of the MIL-L-23699 oil revealed it would readily support fungal and bacterial growth. A survey and test program to determine the microbial susceptibility of the MIL-L-23699 and MIL-L-7808 synthetic engine and transmission lubricants was undertaken. Of the 33 QPL samples of new and used lubricants obtained, only three were found to be resistant to microbial attack. The test microorganisms appeared to attack both the pentaerythritol and trimethylolpropane major base-stock components with equal ease. The three microbially resistant products were all manufactured by one company and microbial resistance was probably due to either a side-effect of the gear load carrying additive employed or its instability in the test system employed. Verification of these findings in actual field testing is recommended.

1. BACKGROUND

A report (1) issued by the U. S. Army Aviation Test Board disclosed that one of the shortcomings found during the flyable storage phase of the test of the OH-58A Helicopter System involved fungus accumulation in the MIL-L-23699A oil which caused a blockage of the oil passage in the 90° gear box. The growth of the fungus plugged the drain holes in the tail rotor transmission sight gage and caused a false oil level indication. Subsequently, Hq., U. S. Army Test and Evaluation Command, Aberdeen Proving Ground, Md., reclassified this shortcoming by the test Agency as a deficiency because of possible damage to the gear box which could result from continued operation if lubrication is insufficient. As a result of a letter request from Hq., TECOM (2), a sample of the MIL-L-23699A oil was evaluated for microbial susceptibility by this Laboratory. The result of this test showed that the MIL-L-23699A oil was extremely susceptible to microbial attack (3).

In order to determine if there are any microbially resistant products presently available in the military supply system and included in the applicable Qualified Products List (QPL), this Laboratory requested and received from the Naval Air Propulsion Test Center, Trenton, N. J. 08628, (4) some 33 samples of new and used gas turbine lubricants conforming to MIL-L-23699B and MIL-L-7808 along with several samples of advanced 5cs lubricants (Specification XAS-2354 candidates). Table I describes the samples tested in this study.

Table 1

Gas Turbine Lubricants Evaluated
for Susceptibility to Microbial Attack

Code No.	DATE PRODUCED MIL-L-23699	BATCH NO. PRODUCTS	MANUFACTURER	BASESTOCK COMPONENTS PE=Pentaerythritol TMP=Trimethylolpropane
33	April 1970	--	No. 10	PE
32	Aug. 1964	5	No. 9	PE
31	Aug. 1968	20	No. 8	PE
30	Oct. 1967	29	No. 7	TMP Major PE Minor
29	Feb. 1965	3	No. 7	TMP Major PE Minor
28	May 1967	17	No. 6	PE
27	Nov. 1971	2	No. 5	PE
26	Feb. 1968	3	No. 4	TMP Major PE Minor
25	Jan. 1968	5	No. 3	PE Major TMP Minor
24	Feb. 1971	6	No. 8	PE Major TMP Minor
23	--	--	No. 3	PE Major TMP Minor
22*	Feb. 1968	3	No. 6	TMP Major PE Minor
21*	Aug. 1968	1	No. 5	PE
20*	Feb. 1968	1	No. 2	PE
19*	Jun. 1969	4	No. 7	PE
18*	May 1971	4	No. 6	PE
17*	Aug. 1971	1	No. 2	PE
16	--	--	No. 1	PE (mono, di and triester)
15	--	--	No. 9	PE
14	--	--	No. 11	PE
13	--	--	No. 11	PE
		MIL-L-7808 Products		
12	Nov. 1967	2	No. 7	TMP
11	Aug. 1972	4	No. 8	TMP
10	May 1971	21	No. 9	Diester
9	Feb. 1969	7	No. 10	Hindered Ester
8	Feb. 1971	93	No. 10	Diester
7	May 1972	2	No. 3	TMP Major Diester
		XAS-2354 Candidates		
6	--	--	No. 11	PE
5	--	--	No. 9	PE
4	Jan. 1971	93	No. 10	PE

Table I (cont'd)

Used Oils

3 (T56 Engine Test)	No. 2 (Used Engine Oil)	PE (mono and diester)
2 (E-715 XAS-2354) (T56 Engine Test)	No. 3 (Used Engine Oil)	PE Major TMP Minor
1 (E-715 XAS-2354) (T56 Engine Test)	No. 3 (Used Gear Box Oil)	PE Major TMP Minor

* Samples received in brown bottles taken from drums submitted by each company. Not available in quart cans at NAPTC.

2. TEST PROCEDURE

The oil samples were evaluated by placing 25 ml each of the test oil and Bushnell Haas (5) medium in screw cap or cotton plugged flasks and inoculated. The inoculum consisted of <u>Cladosporium resinae</u> QM7998 (fungus), <u>Pseudomonas putida</u> QMB1620 (bacterium) and the standard ASTM G-2170 mixed fungal spore inoculum consisting of <u>Aspergillus niger</u> QM386, <u>A. flavus</u> QM380, <u>A. versicolor</u> QM432, <u>Penicillium funiculosum</u> QM391, <u>Trichoderma sp.</u> QM365, <u>Aureobasidium pullulans</u> QM279C, plus <u>Chaetomium globosum</u> QM459. The mixed spore inoculum was used as an inoculum which may provide organisms capable of attacking some of the additives used in oils rather than the oils themselves. The inoculated test flasks were placed on a New Brunswick reciprocating shaker set at approximately 90 revolutions per minute. The flasks were incubated for 6 months at 30C and microbial attack of the oils was determined qualitatively by visual observations for the presence of fungal growth or bacterial turbidity using the scale:

0 = no growth or turbidity
1 = questionable growth or turbidity
2 = very slight growth or turbidity
3 = slight growth or turbidity
4 = moderate growth or turbidity
5 = heavy growth or turbidity

Since a preliminary experiment revealed that some of the oils undergo color, viscosity and other physical changes after autoclaving or by dry heat sterilization, oils used in these tests were not sterilized in order to avoid the possibility of degrading any heat labile component of the oil. Sufficient inoculum was presumably added to each flask to overgrow any natural reservoir of organisms present in the oils. However, most of the flasks inoculated with <u>Pseudomonas putida</u> also showed fungal growth after

6 months incubation, thus indicating most of the susceptible oils contained a residence of fungal spores that eventually overgrew the *Pseudomonas putida* inoculum.

3. OBSERVATIONS AND DISCUSSION

The results of these tests are summarized in Table II and are shown visually in Figures 1-6. Although the photographs showed most of the observations reported in Table II, there are some observations reported in Table II which are not visible in the photographs. The heavy turbidity noted in some of the samples inoculated with *Pseudomonas putida* is not clearly visible in the photographs. The photographs also do not differentiate between microbial growth and oil instability, precipitate formation or other physical-chemical changes taking place in the oils.

Out of the thirty-three oils evaluated, only 3 samples failed to show any signs of microbial attack. Code No. 15 (Fig. 4) for MIL-L-23699, Code No. 10 (Fig. 3) for MIL-L-7808 and Code No. 5 (Fig 2) of the XAS-2354 candidates were the only products which did not support microbial attack. However, all 3 samples showed physical changes under the conditions of the tests. Code No. 15 developed a precipitate of small (1/16 - 1/8" in diameter) orange pellets of crystalline material which deposited on the bottom and sides of the test flasks (see Fig. 4). Code No. 10 developed a noticeable oily orange precipitate and Code No. 5 also developed a heavy pink syrupy-like precipitate which were clearly visible in the original colored photographs of Figs. 2 and 3. It is of particular interest that all three samples showing resistance to microbial attack were products of one company. Another product from this company, Code No. 32 (Fig. 5), supported moderate growth of *C. resinae* and very slight growth of *Pseudomonas*.

Table II
Observations on the Microbial
Susceptibility of MIL-L-23699 and MIL-L-7808
Synthetic Oils and XAS-2354 Candidates
During Six Months Incubation

Microbial Growth Rating*

Code No.	Batch No.	After 7 Days			After 6 Months			Comment
MIL-L-23699 Products		Pseud.	Cl. res.	ASTM	Pseud.	Cl. res.	ASTM	
33	--	1	2	2	5	5	4	
32	5	1	0	0	2	4	0	Oil layer tends to fall to bottom of flask.
31	20	1	2	2	4	4	4	
30	29	2	3	3	4	5	3	
29	3	2	4	3	4	5	3	
28	17	2	3	2	5	3	3	
27	2	3	3	2	3	4	3	
26	3	3	3	2	3	5	4	
25	5	2	2	2	3	4	3	
24	6	2	3	2	4	4	2	
23		3	3	3	4	4	3	
22	3	3	3	1	3	4	2	
21	1	0	2	0	1	3	1	
20	1	1	3	0	3	3	3	
19	4	1	4	2	3	[illegible]	5	

Table II cont'd

Code No.	Batch No.	After 7 Days			After 6 Months			Comments
		Pseud.	Cl. res.	ASTM	Pseud.	Cl. res.	ASTM	
MIL-L-23699 Products								
18	4	3	4	2	5	4	3	
17	1	2	4	2	3	4	3	
16		2	3	2	3	3	2	
15		1	1	1	0	0	0	Orange precipitate in small balls and on side of flask in inoculated and uninoculated control-not microbiological.
14		2	4	1	4	4	2	
13		1	2	2	4	4	3	
MIL-L-7808 Products								
12	2	0	5	5	4*	5	5	
11	4	0	5	5	4	5	5	
10**	21	1	0	1	0	0	0	Orange precipitate
9	7	0	5	5	4	5	5	
8	93	2	3	2	5	4	3	
7	2	0	4	5	4	4	5	
XAS-2354 Candidates								
6		1	1	1	4	5	3	
5		0	0	0	0	0	0	Heavy pink syrupy precipitate
4	93	2	2	2	3	3	3	

**Removed from QPL 10 Sept. 1971

Table II cont'd

Code No.	After 7 Days			After 6 Months			Comments
Products Used Oils	Pseud.	Cl. res.	ASTM	Pseud.	Cl. res.	ASTM	
3	2	0	2	3	0	3	
2	1	4	0	3	4	3	
1	2	4	2	3	4	3	

* = Microbial Growth Rating

0 = No growth or turbidity

1 = Questionable growth or turbidity

2 = Very slight growth or turbidity

3 = Slight growth or turbidity

4 = Moderate growth or turbidity

5 = Heavy growth or turbidity

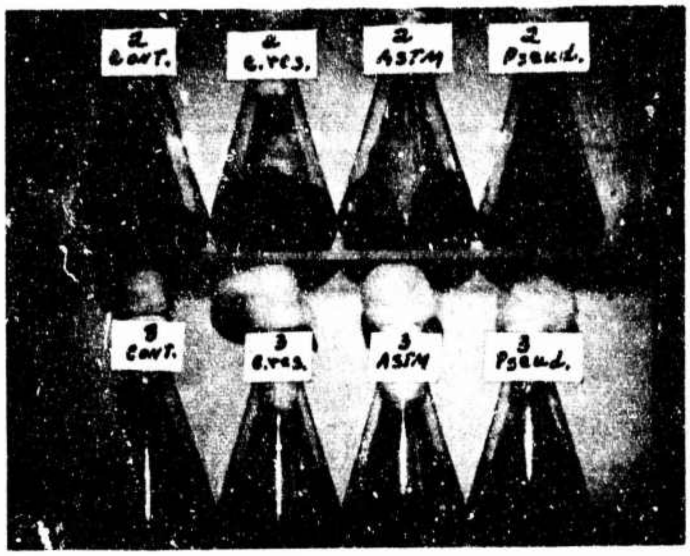

FIGURE 1. Appearance of growth in shake flask test samples of used synthetic gas turbine lubricants Code No. 3 (bottom) and No. 2 (top) after six months incubation. From left to right, first flask represents the uninoculated control, flask two inoculated with Cladosporium resinae, third flask inoculated with ASTM mixed fungal spore inoculum, and last flask inoculated with Pseudomonas putida. Code No. 3 sample contains a mixture of PE and mono and diesters and Code No. 2 sample contains PE as the major basestock component and TMP as the minor component.

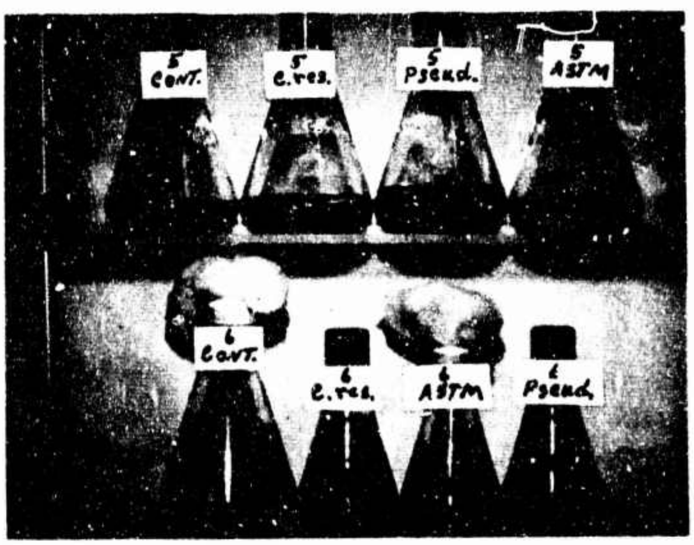

FIGURE 2. Appearance of growth in XAS-2354 synthetic gas turbine lubricants Code Nos. 6 (bottom) and 5 (top) in shake flask tests after six months incubation. From left to right, first flask represents the uninoculated control, flask two inoculated with Cladosporium resinae, third flask inoculated with ASTM mixed fungal spore inoculum, and last flask inoculated with Pseudomonas putida. (Note flasks 3 and 4 of Code No. 5 have been reversed.) Code No. 5 developed a heavy pink syrupy-like precipitate but did not support fungal growth.

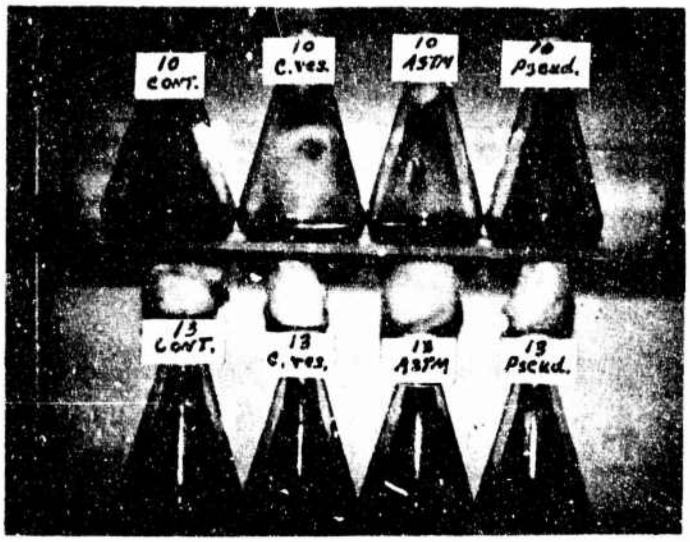

FIGURE 3. Appearance of growth in shake flask test samples of synthetic gas turbine lubricants Code Nos. 13 (bottom) and 10 (top) after six months incubation. From left to right, first flask represents the uninoculated control, flask two inoculated with Cladosporium resinae, third flask inoculated with ASTM mixed fungal spore inoculum and last flask inoculated with Pseudomonas putida. Code No. 10 sample developed an oily orange precipitate but did not support microbial growth.

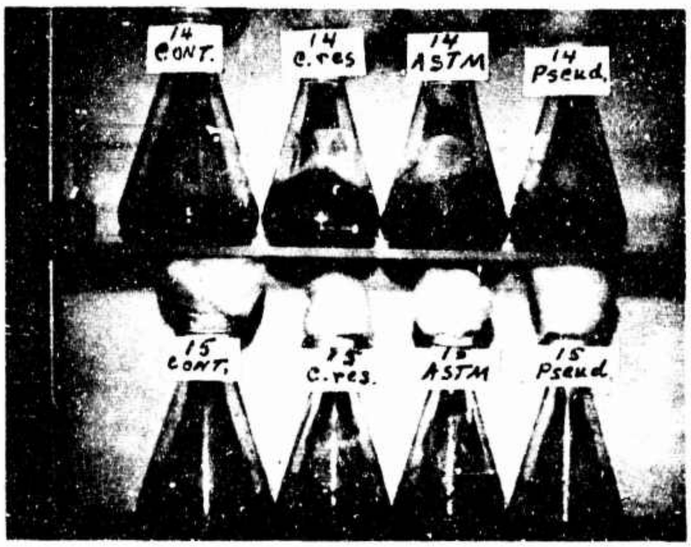

FIGURE 4. Appearance of growth in shake flask test samples of synthetic gas turbine lubricants Code Nos. 15 (bottom) and 14 (top) after six months incubation. From left to right, first flask represents the uninoculated control, flask two inoculated with Cladosporium resinae, third flask inoculated with ASTM mixed fungal spore inoculum, and last flask inoculated with Pseudomonas putida. The orange pellets and crystalline material deposited on the bottom and sides of the flasks containing Code No. 15 sample is a precipitate and not microbiological growth.

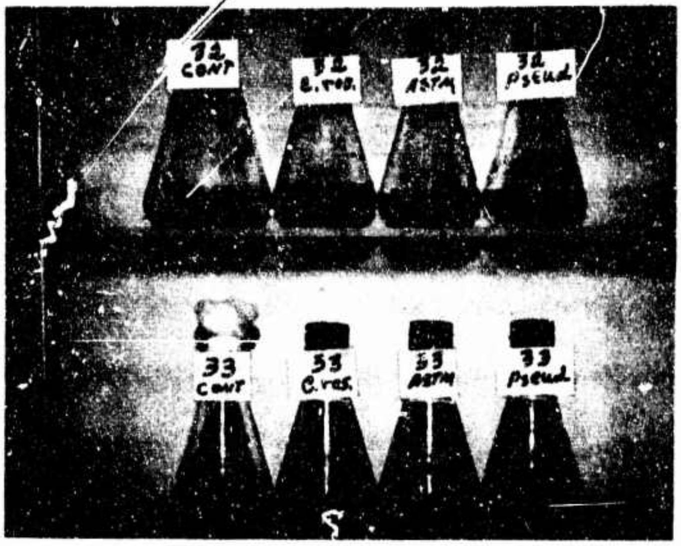

FIGURE 5. Appearance of growth in shake flask test samples of synthetic gas turbine lubricants Code Nos. 33 (bottom) and 32 (top) after six months incubation. From left to right, first flask represents the uninoculated control, flask two inoculated with Cladosporium resinae, third flask inoculated with the ASTM mixed fungal spore inoculum, and last flask inoculated with Pseudomonas putida. The growth and inversion of the oil layer observed in Code No. 32 contain PE as the basestock component.

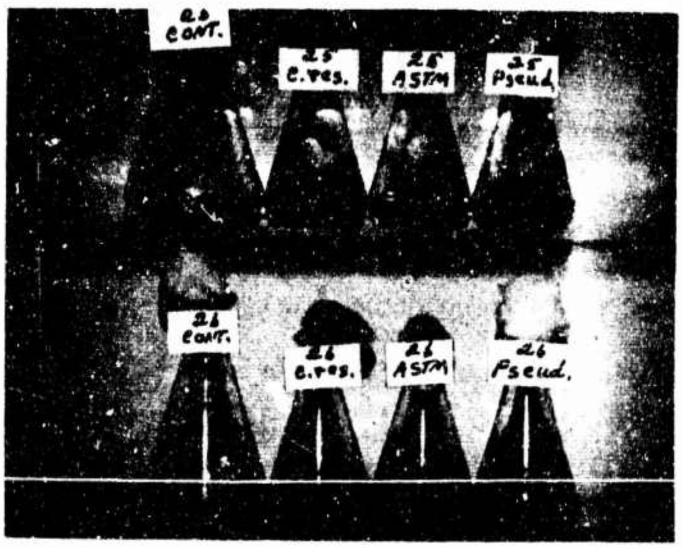

FIGURE 6. Appearance of growth in shake flask test samples of synthetic gas turbine lubricants Code Nos. 26 (bottom) and 25 (top) after six months incubation. From left to right, first flask represents the uninoculated control, flask two incubated with Cladosporium resinae, third flask inoculated with the ASTM mixed fungal spore inoculum, and last flask inoculated with Pseudomonas putida. Code No. 26 contains TMP as the major component and PE as a minor component, whereas Code No. 25 contains PE as the major component and TMP as the minor component. Extensive fungal growth is clearly visible in both samples.

The oil layer tended to drop to the bottom of the flask instead of floating completely on top of the Bushnell-Haas mineral salts solution. This observation may indicate that these oils are formulated so that the specific gravity of the oils slightly exceeds that of water, or the oils either break down or are partially soluble in the presence of water containing minerals (Bushnell-Haas solution). Since there is some lack of stability of these four oils with the Bushnell-Haas solution, this may also influence their accessibility to the microorganisms and therefore account for their resistance to microbial attack. Under another test system using lesser amounts of Bushnell-Haas medium, these seemingly microbially resistant oils may be physically stable and permit microbial growth. The other explanation is that the producer may incorporate a chemical additive that exerts biocidal activity over the test organisms used. The need to know the chemical composition of these oils therefore becomes obvious in order to ascertain why the oils appear to provide resistance to microbial attack while the other products evaluated do not.

The data in Table II do not show any differences in the microbial susceptibility of products composed mainly of pentaerythritol (PE) vs. trimethylolpropane (TMP). Products containing PE or TMP as either a major or minor component appear to be equally susceptible to microbial attack. For example, Code Nos. 30, 26, and 22 all contain TMP as the major component and PE as the minor component as formulated MIL-L-23699 lubricants. The extent of growth noted on these samples is comparable, although not necessarily to the same numerical degree, to that found on samples (Code Nos. 23, 24, and 25) which contain PE as the major component and TMP as the minor component as illustrated by Fig. 6. Pentaerythritol and its mono-, di- and triesters in both new and used oils all appear to support good microbial growth as shown in Figs. 1 and 5. Since formulated oil containing only TMP is not currently available in the system it is not possible to state whether or not such an oil would support microbial growth. Based on the samples evaluated where TMP is the major component, the indications are that it probably would support microbial growth. However, proof of this supposition is still required.

4. CONCLUSIONS

These studies clearly show the majority of the presently available gas turbine lubricating oils are very susceptible to microbial attack. It should be emphasized that certain environmental conditions must be present before microbial growth becomes significant. The presence of water and a warm environment are the two main predisposing environmental conditions which will permit most of these oils to support microbial growth.

Only 3 products of the thirty-three commercial samples evaluated appear to be resistant to microbial attack under the test conditions employed. It should be noted that these resistant products were also unstable in the test flask environment which may account for their resistance of microbial attack. The gear load carrying additive used in the resistant formulations may also have accounted for the lack of growth.

The best way to prevent microbial attack of any natural or synthetic petroleum product is to keep it free of water. Since aircraft utilizing these lubricants are often operated or stored in tropical environments where water of condensation contaminates nearly all vented fuel tanks, removing the water may not always be a practical solution to the problem, although draining of all accumulated water from the oil reservoir tanks should be part of a routine maintenance procedure. An alternative preventive procedure is to take advantage of the oil temperature reached during operation. Since the operating temperature of the helicopter transmission oil normally reaches about 104C, making certain that the lubricant temperature reaches 104C for several minutes as part of a routine maintenance check every 2 to 3 days should prevent microbial and water accumulation in the oil sumps. However, since it is our understanding that there is no oil flow through the tail rotor transmission glass sight gages, operating temperatures within the sight gages may not be hot enough to destroy the microorganisms growing on the inside of the sight gage. Therefore, manual cleaning and sterilization of this area may be required in order to reduce the probable source of continual microbial contamination.

If the water and microbial contamination cannot be practically removed from the oils by any of the above techniques, the use of microbially resistant oils may be required. The 2 microbially resistant oils Code Nos. 15 and 10 presently in the system together with the XAS-2354 candidate oil Code No. 5, should be further tested under field testing conditions in order to verify that they maintain their resistance under field use. Caution should be exercised in the use of these products since it has been established that these oils are not compatible with silicone rubber seals. In certain helicopter engines using silicone rubber seals in the torque metering devices, the resistant formulations have been found to cause the seals to stick which resulted in erroneous torque readings and serious oil leaks (6).

An alternate approach is to add a biocide to the oil formulations to render them resistant to microbial attack. Since the bioresistance of some of the products may be a side-effect of the gear load carrying additive, consideration should be given to determining the biocidal activity (if any) of this additive. It is possible that this additive may have biocidal properties in addition to its load carrying properties similar to the biocidal properties the anti-icing additive used in JP-4 fuel (ethylene glycol monomethyl ether MIL-L-27686) was found to have had.

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